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MICROBIOLOGICAL SAFETY OF FRUIT AND VEGETABLES AT THE PRIMARY PRODUCTION STAGE®

Bezpieczeństwo mikrobiologiczne warzyw i owoców na etapie produkcji pierwotnej®

Consumers are increasingly interested in healthy lifestyles, leading to increased consumption of fresh produce. This may contribute to their exposure to an increased risk of foodborne illness. Before consumption, fresh produce does not undergo processing steps each time that ensure effective removal or inactivation of pathogenic microorganisms. This problem has resulted in a significant increase in reports of disease outbreaks associated with ready-to-eat fruits and vegetables in recent years. In addition, the nature and sources of microbial contamination of these products is critical to the development of appropriate countermeasures with the potential for implementation by food manufacturers. The objective of this review was to characterize the foods most susceptible to microbial contamination and the microorganisms responsible for outbreaks based on information available in scientific publications. Pathogenic strains of Escherichia coli and Salmonella and norovirus accounted for the majority of cases. There is a need for standardized data sets on all aspects of outbreaks. Providing knowledge to food entrepreneurs will allow them to implement better strategies to improve safety of fresh produce.

Key words: fresh vegetables and fruit, microbial contamination, produce outbreaks.

Konsumenci są coraz bardziej zainteresowani zdrowym stylem życia, co prowadzi do zwiększonego spożycia świeżych produktów. Może to przyczynić się do narażenia na zwiększone ryzyko chorób przenoszonych drogą pokarmową. Przed spożyciem świeże produkty nie są za każdym razem poddawane etapom przetwarzania, zapewniającym skuteczne usunięcie lub inaktywację mikroorganizmów chorobotwórczych. Spowodowało to w ostatnich latach znaczny wzrost liczby zgłoszeń ognisk chorób związanych z owocami i warzywami gotowymi do spożycia. Charakter i źródła mikrobiologicznego zanieczyszczenia tych produktów są kluczowe dla opracowania odpowiednich środków zaradczych, które mogą być wdrożone przez producentów żywności. Celem tego przeglądu jest omówienie żywności najbardziej podatnej na zanieczyszczenia mikrobiologiczne oraz mikroorganizmów odpowiedzialnych za powstawanie ognisk chorób w oparciu o informacje dostępne w publikacjach naukowych. Większość przypadków stanowiły patogenne szczepy Escherichia coli i Salmonella oraz norowirusy. Istnieje potrzeba standaryzacji zestawów danych dotyczących wszystkich aspektów ognisk chorobowych. Dostarczenie wiedzy przedsiębiorcom branży spożywczej pozwoli na wdrożenie lepszych strategii w celu poprawy bezpieczeństwa świeżych produktów.

Słowa kluczowe: świeże warzywa i owoce, zanieczyszczenie mikrobiologiczne, ogniska chorób.

INTRODUCTION

Unsafe food has been a health problem for centuries, and many food safety issues are not new. Although governments around the world are making every effort to improve the safety of the food supply, the incidence of foodborne illnesses remains a major health problem in both developed and developing countries. An estimated 1.8 million people die each year from diarrheal diseases, and most of these cases can be attributed to contaminated water or food. Proper food preparation can prevent most foodborne illnesses [40, 60]. In many cases, fresh fruits and vegetables are ready-to-eat (RTE) commodities [37]. Consumers often do not subject these foods to any processing step before consumption (lack of effective removal or inactivation of contaminants). Their increased consumption, combined with globalization and large-scale production of RTE foods [45], has resulted in longer distribution times and increased distribution distances [32]. In particular, product-related disease outbreaks have increased in recent years [26, 45]. Reports from the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) have indicated an increase in the trend in the contribution of non-animal foods to the total burden of foodborne illness in Europe [13, 14, 15]. The same trend has been observed in reports from the US Centers for Disease Control and Prevention (CDC) regarding foodborne illness outbreaks [5].

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GROUPS OF MICROORGANISMS INVOLVED IN FOOD CONTAMINATION

The increase in world population has resulted in an increased demand for food [5, 39]. However, food security problems in different regions of the world arise due to food spoilage [37, 39]. Food spoilage occurs when the quality of food deteriorates from its original organoleptic properties found during processing. Food spoilage causes huge economic losses to both producers (farmers) and consumers [39]. Food spoilage rates are influenced by factors such as storage temperature, water availability, pH, initial microbial load (total viable count-TVC), processing and the most all presence of spoilage-causing microorganisms including bacteria and fungi [4, 38, 39, 41, 43]. A report prepared by the United Nations Food and Agriculture Organization states that one-third of the food produced for human consumption is either spoiled or wasted [23]. Food spoilage is defined as a change in the quality of food that makes it undesirable and unfit for consumption by both humans and animals (unpleasant odor and changes in texture and appearance) [2, 34; 42]. This is a complex process resulting from underlying causes that can be broadly grouped as microbiological, chemical, or physical [48]. In addition to the problems of food insecurity and economic losses [29], spoiled food also contributes to food waste, which is another global environmental problem [24]. Colonization of food by spoilage-causing microorganisms occurs in different ways depending on the type of food. There are countless types of microorganisms that may be involved in food spoilage. A few of these are listed below [11, 39, 47, 49, 57]:

- Mold fungi. These are capable of growing over a wide range of temperatures. Representative types of molds that cause food spoilage include *Rhizopus, Penicillium, Aspergillus, Geotrichum*, *Fusarium*, *Mucor*, *Alternaria, Cladosporium*, *Eurotium,* and *Byssochlamys;*.
- Yeasts. Food spoilage microorganisms may act on perishable foods, indicating that the food has been stored for an extended period of time after synthesis or harvest. a. Fermentation yeasts. Representative genera include *Saccharomyces* and *Zygosaccharomyces*. b. Oxidative yeasts. Representative genera include *Saccharomyces* and *Zygosaccharomyces*. b. Oxidative yeasts. Representative genera include *Mycoderma*, *Candida*, *Pichia*, and *Debaryomyces*. They are involved in food spoilage, which is defined as having oxidative damage;
- *Enterobacteriaceae.* These Gram-negative mycobacteria are facultatively anaerobic, fermentative, mesophilic, non-spore forming, and lack oxidase and catalase. Representative types of these bacteria include *Escherichia*, *Erwinia*, *Enterobacter*, *Citrobacter, Serratia*, and *Proteus*. These bacteria are usually involved in spoilage of fresh vegetables, meat poultry, fish, and eggs;
- *Pseudomonadaceae.*Representative types of this family of bacteria include *Xanthomonas* and *Pseudomonas.* These non-spore-forming Gram-negative mycobacteria are inherently psychrophilic, aerobic and oxidase-positive. Pseudomonads are well known major microorganisms causing spoilage of seafood, fresh meat, poultry and eggs;
- Lactic-acid bacteria. Representative genera are *Lactobacillus*, *Leuconostoc*, *Streptococci*, *Lactococcus*, *Enterococci*, and others. The growth of these bacteria in the same fresh foods, such as meats, vegetable salads, fluid milk, and so forth constitutes spoilage.
- *Neisseriaceae.* Representative genera of this family include *Acinetobacter* (oxidase negative) and *Moraxella* (oxidase positive). Some strains of *Acinetobacter* are psychrophilic. They also infect food, as do pseudomonas;
- *Micrococcaceae.* The two main genera representative of this genus are *Micrococcus* and *Staphylococcus.* They are commonly involved in spoilage of fresh produce and processed meat and poultry [39].

In 2019 by comprehensive review the researchers at Teagasc and National University of Ireland Galway identified the main aetiological agents for foodborne disease outbreaks associated with fruit and vegetables. In this review *Listeria monocytogenes*, followed by *Escherichia coli* and *Salmonella*, were associated with the most deaths [37]. RTE foodstuffs, and particularly fresh fruits and vegetables, are notably vulnerable to contamination by microbial pathogens, and play a key role in pathogen transmission [5]. Also the number of produce related disease outbreaks is increasing worldwide, as is, which has a serious socioeconomic impact. Based on the reviewed scientific literature, the most common foodstuffs associated with outbreaks can be divided into three major groups: leafy green vegetables, sprouted seeds, and fruits (particularly soft fruits and fruit juices) [37]. Understanding the mechanisms of food spoilage is paramount in developing robust food spoilage and waste prevention technologies [43, 44].

SOURCES OF CONTAMINATION

Human pathogen contamination of fresh fruit and vegetables can occur at any number of stages in the production and distribution chain (Figure 1). During cultivation, crop contamination can occur from the soil (e.g. from the use of organic fertilisers). Plants can also be contaminated by animal manure or bird droppings [5]. The application of water is also an important consideration (used for irrigation purposes, for pesticide application and cooling). Contamination can also occur during harvest. Pathogens can be transferred to produce either from the handlers or contaminated equipment (as knives, crates or belts) [37]. During transport, maintenance of the cold chain plays an important role in preventing the proliferation of microorganism, contributing to both product quality and safety [5]. Extreme rain can lead to flooding of terrains or run off events that can result in contamination of crops. On the other hand, extreme drought conditions can lead to the utilization of water of lower microbiological quality, due to the lack of potable water, increasing the chances of contamination (37, 62]. The survival of microorganisms in produce may be dependent on factors such as air temperature, solar radiation, or humidity [37]. Handling of contaminated soil or produce can result in transference of pathogenic microorganisms to workers' hands. Additionally, the survival of microbial pathogens such as *E. coli* O157 and *Salmonella* on nitrile and latex gloves after transfer from contaminated produce has been demonstrated [12], which may increase the probability of further contamination of foodstuffs. Cross contamination may occur due to the transport of microorganisms via washing water, particles present in the water (that is, soil and small plant fragments), or plant to plant contact [22, 37].

Fig 1. Potential routes of microbial contamination in crop production.
Rys. 1. Potencjalne drogi skażenia mikrobiologicznego w produkcji roślinnej.
Source: Own elaboration based on source Machado-Moreira et al. [37]
Źródło: Opracowanie własne na podstawie Machado-Moreira et al. [37]

ENSURING PRODUCE SAFETY

Countries also have different criteria regarding acceptable parameters for water and produce quality [5]. Sprouted seeds are a particular source of risk, given the potential of contaminated seeds to cause contamination of the product, leading to microbial disease outbreaks. In Europe, there is no standardized approach in terms of microbial decontamination of seeds (each member state determining and authorizing specific treatments) [37]. Seeds should be treated in order to ensure their microbiological quality. Different treatment options are available [56] e.g. high chlorine dosages, the use of organic acids, different chlorine-based chemical compounds, electrolyzed water, ozone, and more recently, atmospheric cold plasma discharges or plasma-activated water [7, 30, 53]. Physical approaches, such as thermal inactivation of microorganisms, irradiation, high pressure, or UV treatments, can also be employed or biological alternatives, such as the usage of bacteriophages, bacteriocins, or protective cultures, have also been suggested as seed treatment alternatives [37, 51, 56]. The maintenance of cold storage conditions throughout processing, shipping, storing, and retail of fresh produce is of crucial importance, as low temperatures will hinder the proliferation of bacterial pathogens during these steps [8]. One challenge to selecting suitable treatments is the ease with which some products (soft fruits) can be damaged. Another factor is that a demonstration of lab – based efficacy is no guarantee of success in real world scenarios, as numerous additional interfering factorswill also be at play in th field. The regulatory landscape also need to be acknowledged and can vary globally [5].

COVID-19 AND FRESH FOOD SAFETY

It is unlikely that people can contract COVID-19 from food or food packaging. COVID-19 is a respiratory illness and the primary transmission route is through person-toperson contact and through direct contact with respiratory droplets generated when an infected person coughs or sneezes [6, 9, 17, 27, 33, 35, 46, 61]. There is no evidence to date of viruses that cause respiratory illnesses being transmitted via food or food packaging. Coronaviruses cannot multiply in food; they need an animal or human host to multiply [17]. Despite this, some consumers perceive there is a risk of COVID-19 infection resulting from open food displays. It is important to maintain good hygiene practices around open food displays, such as salad bars, fresh produce displays, and bakery products. Consumers should always be advised to wash fruit and vegetables with potable water before consumption. Both customers and staff should strictly observe good personal hygiene practices at all times around open food areas [17]. Resent research evaluated the survival of the COVID-19 virus on different surfaces and reported that the virus can remain viable for up to 72 hours on plastic and stainless steel, up to 4 hours on copper, and up to 24 hours on cardboard [59]. This research was conducted under laboratory conditions (controlled relative humidity and temperature) and should be interpreted with caution in the real-life environment [5]. It is imperative for the food industry to reinforce personal hygiene measures and provide refresher training on food hygiene principles to eliminate or reduce the risk of food surfaces and food packaging materials becoming contaminated with the virus from food workers [10]. Personal protective equipment (PPE) can be effective in reducing the spread of viruses and disease within the food industry, but only if used properly [37]. The food industry is strongly advised to introduce physical distancing and stringent hygiene and sanitation measures and promote frequent and effective handwashing and sanitation at each stage of food processing, manufacture and marketing [17].

OUTBREAKS

Bacteria, virus, and parasites have all been implicated in RTE produce–related disease outbreaks (Table 1) [37]. Norovirus and Hepatitis A (HAV) are responsible for a significant number of reported cases of foodborne infection [1, 20, 23, 28, 50]. Norovirus (excreted in high number in human feces, and transmitted via the fecal–oral route) is linked to the majority of reported outbreaks (304 outbreaks, 53.2% of total) and infections (27,495 cases, 37.7% of total reported). They are present in several groundwater sources [3, 19]. HAV has been shown to be internalized by green onions and spinach, in static hydroponic culture and nutrient film technique culture [51]. In Machado-Moreira et al. [37] study, leafy green vegetables, responsible for 34.8% of cases and 72.4% of outbreaks [16, 55], and soft fruits (55.7% of cases) [1, 50] are the main foodstuffs implicated in the transmission of norovirus. *Escherichia coli* is responsible for a high number of reported cases, accounting for 16 416 infections. Nonpathogenic strains of *E. coli* are common inhabitants of the gastrointestinal tract of humans and animals, but some strains are virulent and capable of causing disease to the gastrointestinal, nervous and urinary systems (such as *E. coli* O157:H7, *E. coli* O96, *E. coli* O104 and *E. coli* O121). *Salmonella* is responsible for over 20% of all reported cases in this study. *Shigella*, *Campylobacter*, *Bacillus*, and *Listeria* are the other bacterial genera identified as relevant to RTE food–related outbreaks. Of the pathogens analysed in Machado-Moreira et al. [37] study, *Listeria monocytogenes* had the highest mortality rate (19%); [18, 25, 36, 54, 58]. It was responsible for the outbreak with the second highest mortality count (33 deaths out of 147 disease cases) included in this study, which resulted from the consumption of contaminated cantaloupes in the United States in 2011 [36]. Outbreaks whose setting was known were mainly associatedwith commercial food services (canteens, restaurants, market stalls, catered events, hotels, or cruise ships) and private consumption of contaminated foodstuffs or supermarket distribution. It is worth noting that based on the analyzed scientific publications no setting information was available for the majority of considered occurrences (64.6%, 369 out of 571 outbreaks) [37].

FUTURE PREVENTION

Implementing strategies to prevent or minimize microbial contamination of RTE foods controls the burden of disease caused by foodborne microorganisms. Several interventions have been extensively reviewed in the scientific literature [21, 31]. Food producers and processors play a key role in minimizing contamination events and/or implementing strategies to mitigate this issue across the farm-to-fork chain. It is crucial that producers and processors are provided with relevant data regarding fresh produce–related microbial disease outbreaks, covering aspects including the causative

Table 1. Relevant microorganisms implicated in RTE produce related outbreaks in developed countries, as identified in scientific publications*

Tabela 1. Istotne mikroorganizmy zidentyfikowane w publikacjach naukowych, które wystąpiły w ogniskach związanych z produktami gotowymi do spożycia w krajach rozwiniętych*

Cases	Outbreaks	Deaths
Norovirus (27 495)	Noravirus (304)	*L.monocytogenes* (83)
E.coli (16 416)	*Salmonella* (128)	*E.coli* (60)
Salmonella (15 137)	*E.coli* (41)	*Salmonella* (24)
Cyclospora (5 235)	Hepatitis A (23)	Hepatitis A (5)
Hepatitis A (4 804)	*Cyclospora* (22)	*Yersinia* (1)
Shigella (1 139)	*L. monocytogenes* (13)	
Yersinia (686)	*Shigella* (12)	
Cryptosporidium (640)	*Yersinia* (6)	
L. monocytogenes (436)	*Giardia* (6)	
Campylobacter (360)	*Cryptosporidium* (5)	

*Data obtained from 1980-2016 *Dane uzyskane w latach 1980-2016

Source: [37]

Źródło: [37]

microbial pathogen and the vehicle of contamination. Information regarding produce-related outbreaks is available from a number of sources (health protection agencies, such as CDC, EFSA and HPSC and PHE) [37, 52]. Gil et al. [21] and Julien-Javaux et al. [31] have compiled very comprehensive lists of measures to mitigate microbial contamination of crops (primary production). Researchers highlighted aspects such as previous land usage, proximity to livestock producing facilities or industrial activities, and terrain characteristics (potential for water and soil runoff) as key aspects to be taken into account from a food safety perspective [21, 37]. The importance of different water sources and irrigation methods and their associated microbial risks is also taken into consideration [5, 37]. In addition to microbial contamination, horticultural crops are also at potential risk of chemical contamination. It can be observed a drive to minimise the use of chlorine, owing to potential health implications of chlorine by products such as chlorates, environmental concerns, the implementation of maximum residue limits, and consumer concerns about the use of chemicals in the food supply chain [5, 37]. A careful balance is therefore required to provide assurance of microbial safety in horticultural production. A multidisciplinary approach is needed to address produce safety, for example through projects such as the HortAssure project funded by the Irish Department of Agriculture, Food and the Marine (DAFM) [5] that will help to facilitate the safe consumption of fresh vegetables and fruit as part of healthly diet.

CONCLUSION

As consumer demand for fresh food increases, so does the need to ensure its quality and safety. Microorganisms that cause food spoilage, including bacteria, fungi, and molds, play an important role in this process regardless of its source [5, 37, 62]. Storage temperature, processing procedures, and transportation in the supply chain also contribute to adverse changes in food. While bacteria are more prevalent in meat spoilage, fungi and molds cause spoilage in fruits and vegetables. To prevent this, effective strategies, policies, and technology that can be applied at the processing and storage stages should be developed and used to reduce the growth of microorganisms [5]. Early detection of microorganisms that cause food spoilage should be pursued (metabolomics and metagenomics, including next generation sequencing and whole genome sequencing) [5, 37]. It is also suggested to use hurdle technology, which can extend the lag phase of microorganisms, and predictive microbiology to predict the growth rate of food spoilage-causing microorganisms.

PODSUMOWANIE

Wraz ze wzrostem zapotrzebowania konsumentów na świeżą żywność, pojawia się potrzeba zapewnienia jej jakości i bezpieczeństwa. Mikroorganizmy powodujące psucie się żywności, w tym bakterie, grzyby i pleśnie, odgrywają istotną rolę w tym procesie niezależnie od jej źródła pochodzenia [5, 37, 62]. Temperatura przechowywania, procedury przetwarzania i transport w łańcuchu dostaw również przyczyniają się do niekorzystnych zmian w żywności. Podczas, gdy bakterie są bardziej rozpowszechnione w psuciu się mięsa, grzyby i pleśnie powodują psucie się owoców i warzyw. Aby temu zapobiec należy opracować i wykorzystać skuteczne strategie, politykę i technologię, które mogą być stosowane na etapie przetwarzania i przechowywania, w celu ograniczenia wzrostu mikroorganizmów [5]. Należy dążyć do wczesnego wykrywania mikroorganizmów powodujących psucie się żywności (metabolomika i metagenomika, obejmująca sekwencjonowanie następnej generacji i sekwencjonowanie całego genomu) [5, 37]. Sugeruje się również wykorzystanie technologii płotków, które mogą przedłużyć fazę opóźnienia drobnoustrojów oraz mikrobiologii predykcyjnej do przewidywania tempa rozwoju mikroorganizmów powodujących psucie się żywności.

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